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14. ABSTRACT Multiple sclerosis (MS) is an inflammatory-mediated demyelinating disease of the human CNS. The clinical disease course is variable and starts with reversible episodes of neurological disability (relapsing–remitting (RR-MS) stage). This transforms into a disease of continuous and irreversible neurological decline. Phosphodiesterase (PDE)-4/7 inhibitors can prevent injury-induced reductions of cAMP as well as facilitate tissue protection, anatomical repair, and functional recovery. PDE inhibitor containing nanoparticles (NP), surface modified with peptides that recognize proteins extravasated at sites of vascular disruption (clotting factors, ECM), can accumulate at regions of CNS demyelination, reducing tissue injury and promoting remyelination repair at very low drug doses. Characterization of non-functionalized polymeric (poly(ethylene glycol-b-ε-caprolactone)) NP revealed a size range of 28-36 nm when polymer or solvent concentrations were changed. Fixed solvent (28.57%) and polymer concentrations (3 mg) were chosen for further studies. NPs allowed inclusion of fluorescent dyes (Dil, DiO) and aminated PEG-b-PCL. Peptides (i.e. NQEQVSP, DPEAAE and NIDPNAV) can be conjugated to the aminated PEG-b-PCL. PEG-b-PCL NPs can readily encapsulate PDE inhibitors (Rolipram and BRL-50481), when the drugs are used alone or in combination with Dil dye and the release kinetics of the NPs in solution show slow release up to 28 days. In an experimental EAE model of RR-MS, we demonstrate that non-functionalized Dil NPs fail to accumulate at sites of EAE lesions following systemic administration, at a time just prior to the appearance of pathological changes and behavioral deficits. In contrast, delivery of Dil laden, laminin- or fibrinogen-functionalized NPs, were found to be sequestered to CNS lesions. Delivery of functionalized NPs encapsulating Rolipram, when delivered prior to disease onset, appears to reduce the day of disease onset as well as decrease the severity of the disease score compared to non-targeted, Rolipram-laden NP controls.					
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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory-mediated demyelinating disease of the human central nervous system. The disease usually strikes young adults and typically begins with neurological deficits, variable periods of remission and unpredictable but clinically reversible relapses. This remitting-relapsing RR-MS stage of the disease can persist for 10–15 years and is often followed by a course of continuously progressive neurological disability referred to as secondary progressive MS SP-MS (1-2). A hallmark pathophysiological response to CNS injury or disease is a dramatic reduction in levels of the ubiquitous second messenger, cyclic adenosine monophosphate, cAMP (3), a critical cellular component responsible for regulating vital intracellular functions that include cell metabolism, proliferation, survival and differentiation (4). Levels of cAMP in cells can be reduced by a family of enzymes called phosphodiesterases (PDE) (5-6). It has been shown that the use of a PDE inhibitors prevents injury-induced reductions in cAMP after acute CNS insults (3, 7) as well as facilitates significant tissue protection, anatomical repair, and functional recovery (3). However, despite such promise, dose-limiting side effects, primary of which are pronounced nausea and vomiting, have hampered their clinical development. Both the dose and multi-organ effects of PDE inhibitors could be reduced significantly, if the agent's release is restricted to the microenvironment of tissue pathology. To accomplish this goal a delivery system will be developed in which PDE inhibitors, encapsulated within polymeric nanoparticles, will be specifically targeted to regions of vascular disruption through surface peptide sequences on the nanoparticles that recognize extracellular matrix (ECM) proteins or clotting factors which are extravasated on damaged blood vessels (8-9).

BODY

The ensuing reportable outcomes and milestones combine data sets from both the Primary PI (Damien D. Pearse) and the Partnering PI (Paul Dalton). In line with the SOW, Study Objective responsibilities are allocated to each PI as indicated by surname (Pearse or Dalton). In accordance with the SOW, results and milestones are presented by Study Objective in numerical order with associated tasks listed beneath.

Study Objective 1: Prepare tNPs with surface-modified peptide incorporation [Dalton]

Task 1 (Month 1-3): Synthesize peptides and obtain NP reagents

1.a. Synthesize ECM binding peptides

1.b. Formulate Nanoparticles (NPs)

The synthesis of ECM binding peptides and the successful formulation of NPs were completed and discussed in the Annual Report for Year 1.

Task 2: Surface modify NPs with peptides

2.a. (Month 4-9) Incorporate different quantities of peptides into NPs and test binding efficiency

2.b. (Month 4-12) Optimize binding and maximum peptide incorporation through NH₂-PEG-PCL inclusion

2.c. (Month 4-12) Determine the protein adsorption on the NPs by incubation with fluorescent proteins.

These tests were performed and reported in the Annual Report for Year 1.

2.d. (Month 4-12) Organize obtained chemistry data and discuss final formulations before proceeding

2.e. (Month 4-12) Prepare significant quantities of the different types of tNPs for in vivo testing [Note: tasks 2a-d will be repeated for each peptide with sequential stock production to allow for in vivo testing to take place within 4 months of project initiation]

These tasks were accomplished and reported in the Annual Report for Year 1.

Milestone 1: The production of characterized tNPs for each targeted protein

We demonstrated the ability to generate tNPs and completed the characterization of all three active particles as well as appropriate controls. Milestone 1 was accomplished in Year 1.

Study Objective 2: Screen tNPs for peptide modification that provides maximal NP targeting to EAE lesions [Pearse]

Task 3 (Month 1-3): *Project organization and regulatory permissions*

3.a. *Submit and obtain animal approval*

3.b. *Plan and schedule studies*

Task 4 (Month 4-9): *Identify the temporal window of targeted protein production/presence after EAE*

4.a. *Purchase and acclimatize animals*

4.b. *Induce EAE in the animals*

4.c. *Perfuse animals and dissect tissues at different timepoints*

4.d. *Quantitatively measure protein production/presence by immunohistochemistry and immunoblot analysis*

Tasks 3 and 4 were completed in Year 1 and results were provided in the Annual Report for the different tNPs evaluated in the experimental MS model. Using immunoblotting and immunochemistry, the temporal expression of Nidogen/Laminin and Fibrinogen/Factor XIIIa was determined. These molecules were highly expressed in the lumbar cord at 5-15 days post-induction, particularly in and around perivascular lesions. In contrast, Versican/Tenascin C exhibited a lower expression and a wider distribution along the lumbar cord axis.

Task 5 (Month 7-15): *Test tNPs in an experimental MS model, EAE*

5.a. *Purchase and acclimatize animals*

5.b. *Induce EAE in the animals and provide fluorescent dye encapsulated tNPs (or unmodified NP controls)*

5.c. *Perfuse animals and dissect tissues*

5.d. *Quantify the level of tNP accumulation at the site of the demyelination lesion*

5.e. *Identify those tNPs that did not exhibit target ligand binding following direct injection and reformulate in vitro for re-testing in vivo*

5.f. *Re-test any reformulated tNPs*

Task 6 (Month 10-15): *Organize obtained in vivo targeting data*

6.a. *Graph data and perform statistical analyses*

6.b. *Identify the optimal targeting peptide(s) for future testing of PDE delivery*

6.c. *Discuss ensuing studies, identify abstracts or manuscripts that can be prepared and patents that can be submitted*

We discussed previously in the Year 1 Annual report, the comparative accumulation of Dil laden tNPs with the different ECM binding peptides - Nidogen/Laminin, Fibrinogen/Factor XIIIa and Versican/Tenascin C. While the examination of lumbar spinal cords from EAE animals receiving non-targeted, Dil laden NPs failed to show fluorescent NPs within tissue sections, Nidogen/Laminin directed particles were found localized throughout regions of BBB permeability, immune cell infiltration and tissue damage after EAE and i.v. infusion (Figures 1, 2). We found a similar pattern of spinal cord accumulation with Fibrinogen/Factor XIIIa, but not Versican/Tenascin C, directed particles (Figure 1).

The discussion of abstracts and manuscripts from this work is presented at the end of the report under Task 11.

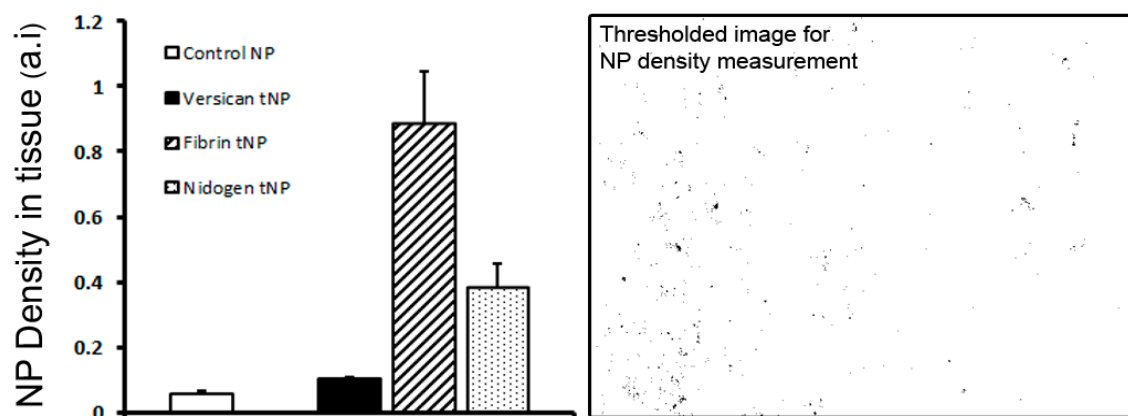


Figure 1: Measurement of Dil NP particle density in spinal cord tissue after EAE and infusion of different tNPs. Both fibrinogen and nidogen peptide functionalized Dil-laden tNPs show significant accumulation in lumbar spinal cord tissue.

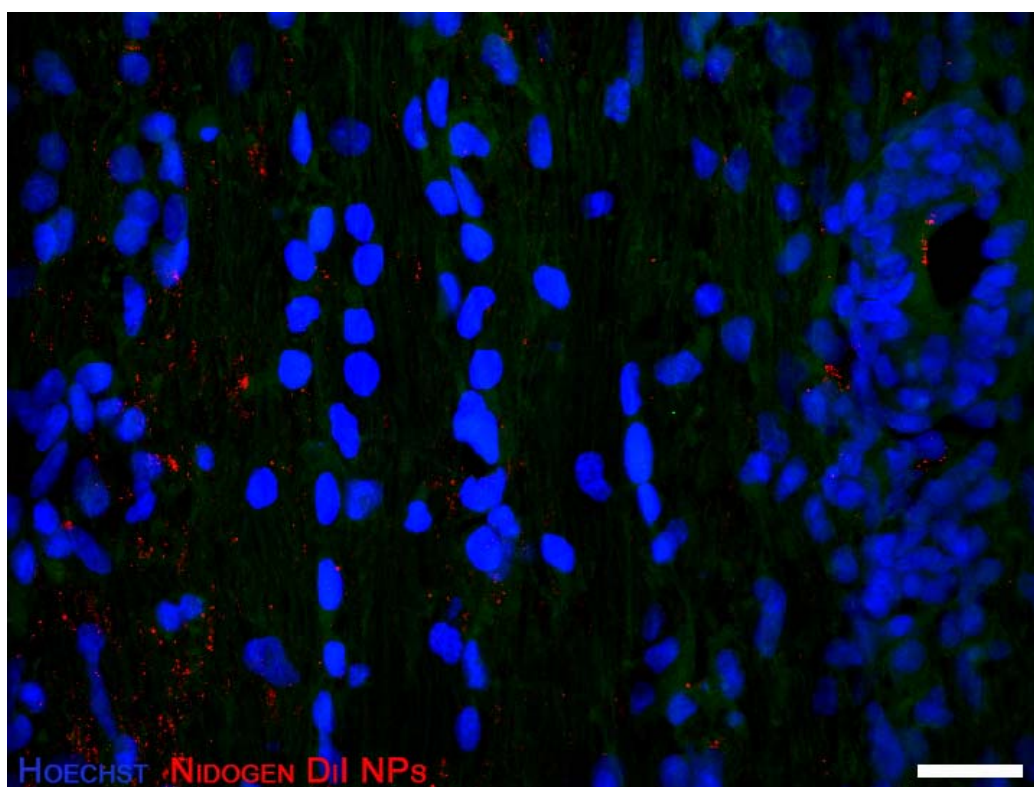


Figure 2: Localization of tNPs within the lumbar spinal cord after EAE and tNP infusion for 2 days.

These results confirmed our hypothesis that targeting of NPs to lesions of the lumbar spinal cord after EAE induction can be achieved when targeting peptides are upon their surface; this has been demonstrated using laminin/nidogen and fibrinogen/factor XIIIa tNPs.

Milestone 2: Identified the optimal peptide(s) for targeting NPs to sites of CNS demyelination

We confirmed our hypothesis that targeting of NPs to lesions of the lumbar spinal cord after EAE induction can be achieved when they are functionalized with peptides upon their surface (non-targeted NPs do not accumulate at these lesions); this has been demonstrated using laminin/nidogen and fibrinogen/factor XIIIa tNPs. Versican/tenascin-C tNPs did not demonstrate good targeting capacity. This could be related to a low and disperse expression of the substrate after EAE or to poor binding efficacy of the peptide. The ensuing efficacy studies using drug laden tNPs (Objectives 3 and 4) continued to employ all three tNPs (versican/tenascin-C tNPs were used as an additional peptide

control) to determine whether tNPs could provide anatomical and functional benefit in an experimental model of MS.

Study Objective 3: Prepare tNPs with the encapsulation of PDE inhibitors or interferon-1a [Dalton]

Task 7: Synthesize peptides and obtain NP reagents

7.a. (Month 10-12) Synthesize ECM binding peptides

7.b. (Month 7-12) Formulate starPEG(sPEG) and PCL NPs

7.c. (Month 13-18) Surface modify NPs with optimal peptide(s)

Task 7 is the same as Tasks 1 and 2 (above) that were described in the Year 1 Annual Report. The three functionalized tNPs (for laminin, fibrinogen and tenascin-c) and their scrambled controls were characterized at different PEG-b-PCL ratios (PEG: 1, 5, 10 and 25%).

Fibrinogen nanoparticles				Laminin nanoparticles				Versican nanoparticles			
	size	charge	pdi		size	charge	pdi		size	charge	pdi
NP	27.77±0.38	0.07±0.55	0.16±0.014	NP	27.77±0.38	0.07±0.55	0.16±0.014	NP	27.77±0.38	0.07±0.55	0.16±0.014
1% tNP	29.91±0.55	-1.45±0.42	0.15±0.009	1% tNP	28.73±0.68	-1.28±0.66	0.33±0.062	1% tNP	27.63±0.42	1.58±0.57	0.19±0.02
5% tNP	30.63±0.45	0.57±0.65	0.19±0.023	5% tNP	29.98±1.34	0.48±1.52	0.17±0.018	5% tNP	26.81±0.33	-1.63±1.27	0.24±0.034
10% tNP	31.19±1.15	0.14±0.84	0.19±0.006	10% tNP	29.42±0.77	0.85±0.91	0.36±0.042	10% tNP	33.30±1.32	0.33±0.7	0.17±0.009
25% tNP	32.1±0.44	-0.23±0.44	0.15±0.012	25% tNP	33.19±0.45	-0.85±0.66	0.18±0.02	25% tNP	35.73±1.94	-0.77±0.84	0.17±0.01
1% scr. tNP	28.36±0.35	-0.16±0.88	0.21±0.015	1% scr. tNP	27.75±0.65	-0.45±0.51	0.15±0.021	1% scr. tNP	28.64±0.85	1.81±0.64	0.17±0.025
5% scr. tNP	27.6±0.39	0.47±0.64	0.18±0.005	5% scr. tNP	27.88±0.49	-0.26±0.99	0.18±0.0196	5% scr. tNP	27.24±0.69	-1.47±0.83	0.27±0.026
10% scr. tNP	29.13±0.29	0.83±0.69	0.15±0.005	10% scr. tNP	30.14±0.35	-2.06±0.81	0.15±0.008	10% scr. tNP	34.63±0.91	-1.08±1	0.14±0.009
25% scr. tNP	35.86±0.77	1.18±0.49	0.11±0.002	25% scr. tNP	38.19±0.64	-0.17±0.3	0.12±0.015	25% scr. tNP	38.3±1.41	0.49±0.51	0.16±0.013

Figure 3: Characterization of functionalized tNPs. Incorporation of aminated PEG-b-PCL led to an increase in NP size.

Task 8: Incorporation of PDE inhibitors or interferon-1a into tNPs

8.a. (Month 7-18) Incorporate different quantities of drugs and test release kinetics

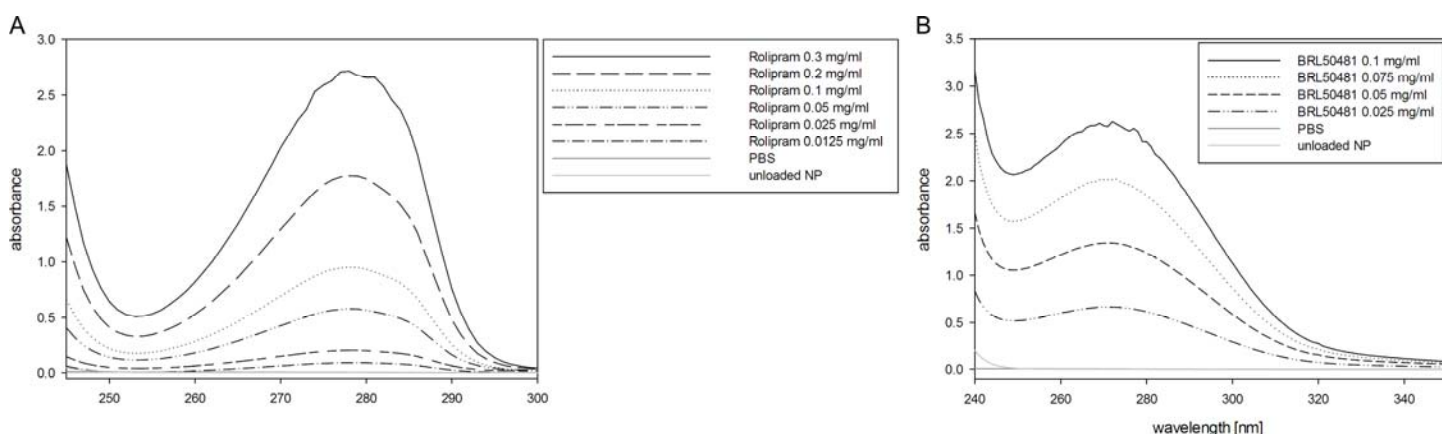


Figure 4: Absorbance spectra of Rolipram (A) and BRL50481 (B).

To measure the amount of drug taken up by the nanoparticles a dilution series of the two different PDE-inhibitors Rolipram and BRL50481 was performed and the absorbance spectra measured (Figure 4). The drug content was calculated at a wavelength of 280nm.

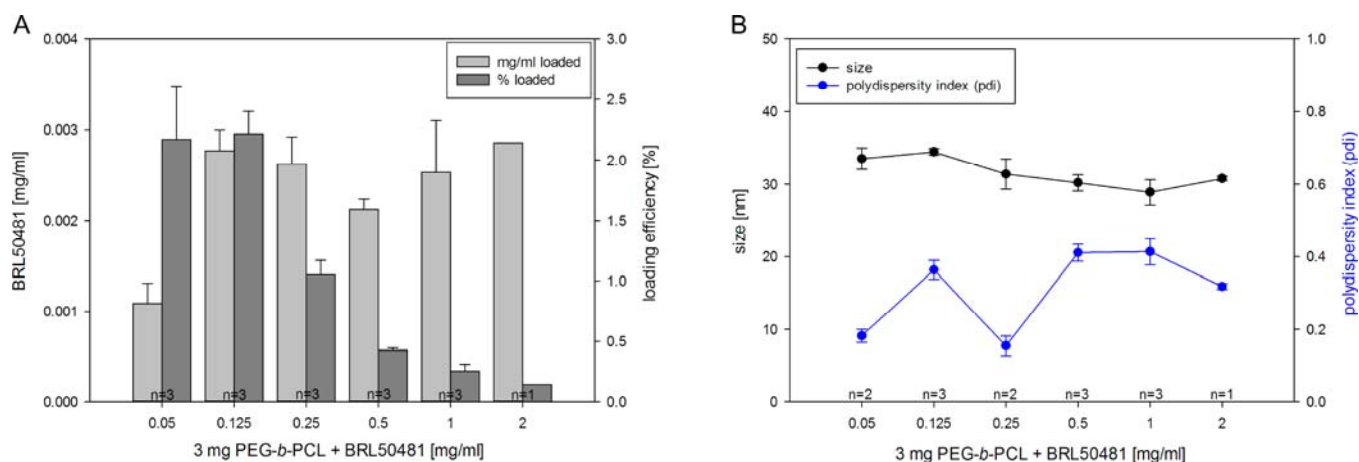


Figure 5: Loading efficiency decreases with increasing drug concentration. A: Drug loading content and efficiency of 3 mg polymer with different drug concentrations. B: NP size did not differ much with different drug concentrations, however the pdi varied, with no clear indication of a concentration effect.

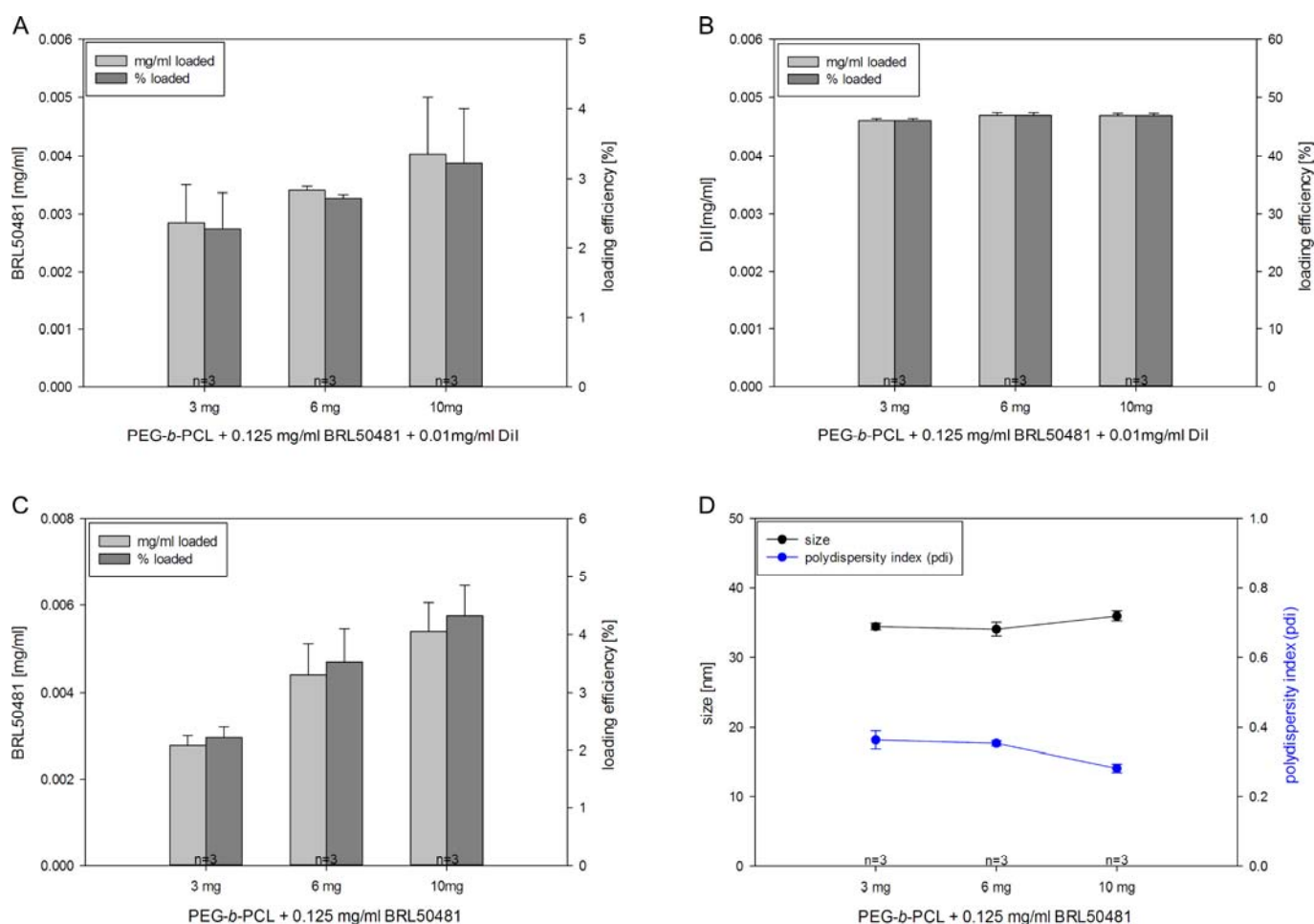


Figure 6: Drug (A, C) and Dil (B) loading content and efficiency of BRL50481 (0.125 mg/ml). Double loading with BRL50481 and Dil did not significantly change the amount of molecules incorporated. D: Drug laden NP size did not change when higher amounts of polymer were used.

The total amount of drug incorporated differed not much when drug concentrations above 0.05 mg/ml were used (0.021 – 0.029 mg/ml), however the loading efficiency decreased accordingly (Figure 5A). Additionally there was no effect of drug concentration on NP size (29-35 nm, Figure 5B). Some variations could be observed for the pdi (0.15-0.65), however no clear trend was noticeable (Figure 5B). Therefore further investigations were performed with lower drug amounts (0.125 mg/ml). The amount of BRL50481 could be increased, when higher concentrations of polymer were used (from

0.0028 to 0.0054 mg/ml, Figure 6A, C). No change of drug loading was observed comparing NP with or without Dil (Figure 6A, C). Similar Dil loading efficiency was comparable to non-drug-laden NP (Figure 6B). As observed before for empty NP no change in drug-laden NP size was observed when polymer concentrations were changed (Figure 6D).

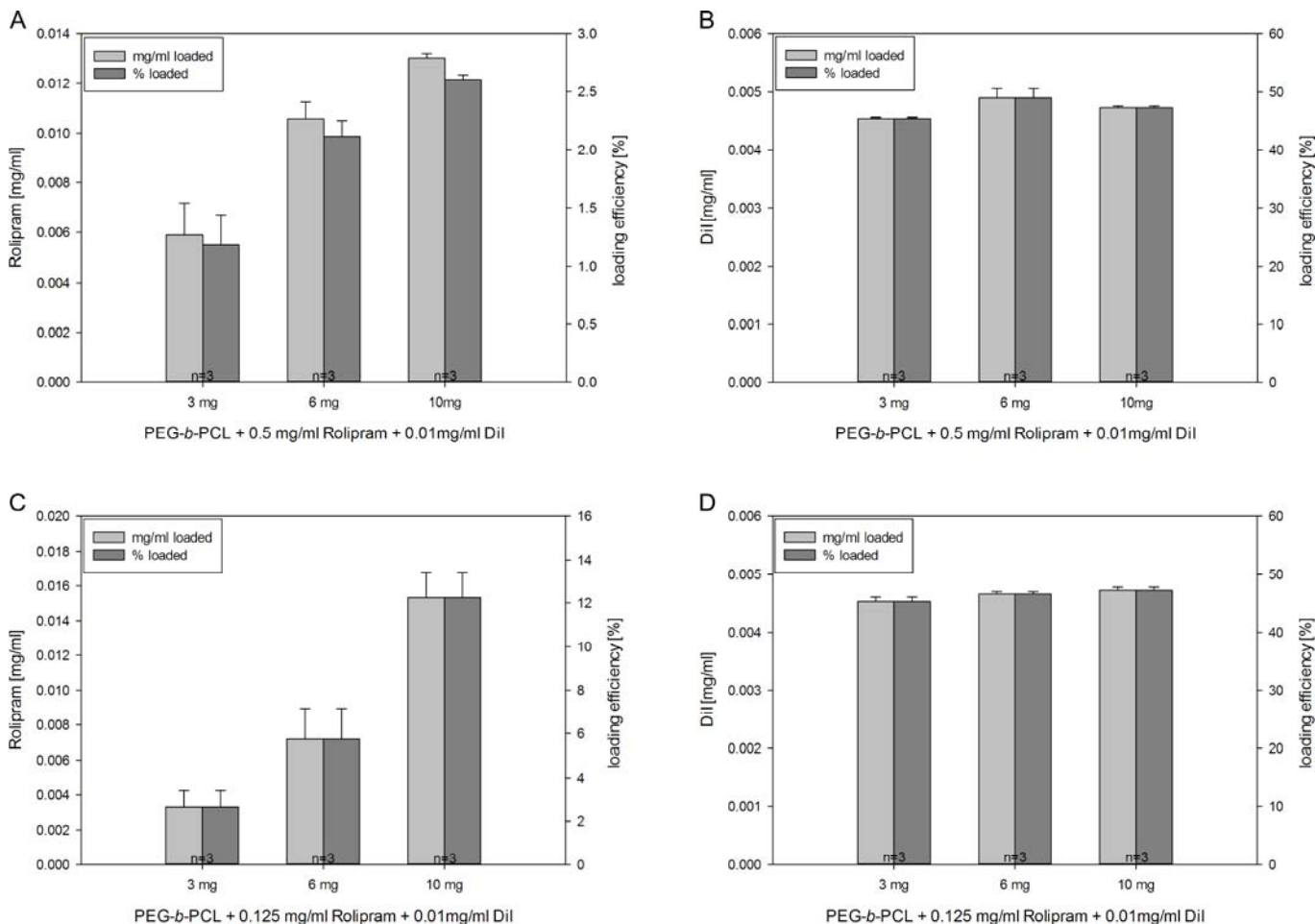


Figure 7: Drug (A, C) and Dil (B, D) loading content and efficiency of Rolipram; at different concentrations of polymer with 0.5 (A, B) and 0.125 mg/ml Rolipram (C, D).

Similar to BRL50481, only low amounts of Rolipram were incorporated by the NP (Figure 7A, C). However the amount could be increased when higher concentrations of polymer were used (from 0.0033 to 0.0153 mg/ml, Figure 7A, C). Lower concentrations of Rolipram (0.125 mg/ml) resulted in similar amounts incorporated into the NP, but increased the loading efficiency (12.25 compared to 2.6%, Figure 7A, C). No change of Dil loading was observed without any or different amounts of Rolipram were used (Figure 7B, D).

8.b. (Month 10-18) *Identify the drug loading concentration that provides slow release kinetics and sustained delivery for 4 weeks in vitro*

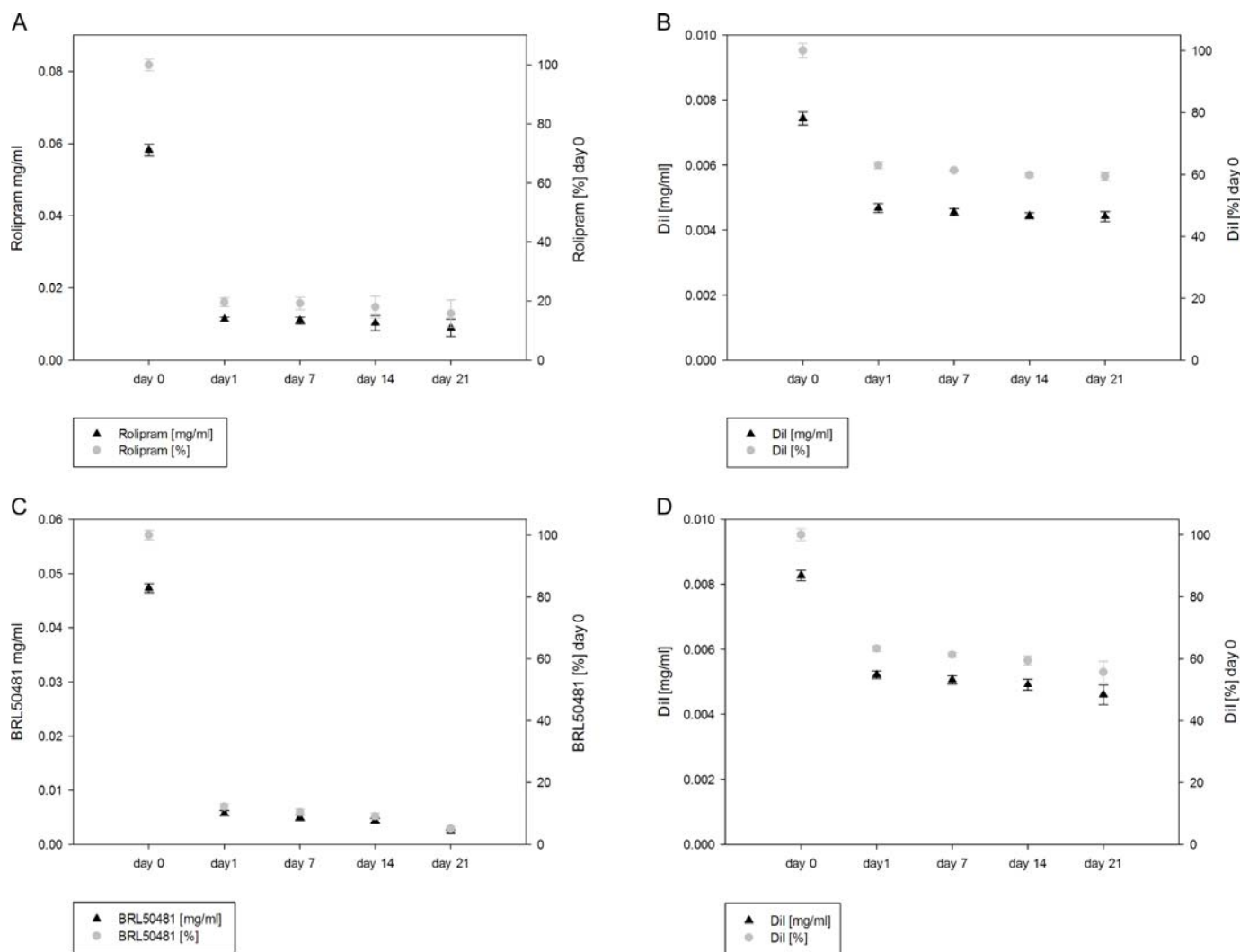


Figure 8: Drug release at 37°C. After an initial burst release, Rolipram (A, B) and BRL50481 (C, D) were slowly released over 3 weeks.

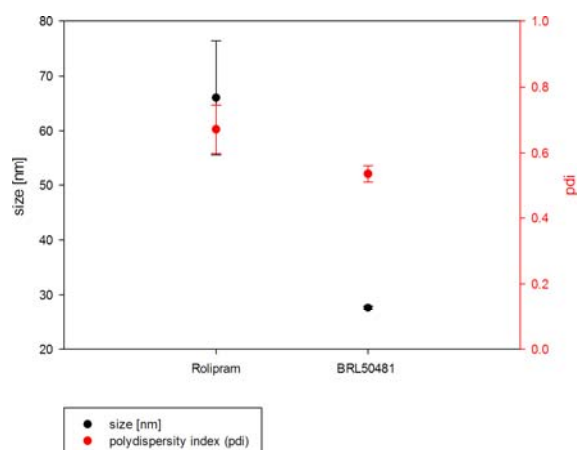


Figure 9: NP size of Rolipram- and BRL50481-laden NP, after four weeks at 37°C.

Both PDE-inhibitors as well as Dil were slowly released over a time period of three weeks, after an initial burst release (Figure 8). The burst release is probably due to surface bound molecules. Dil release was similar regardless of which drug was additionally incorporated (Figure 8B, D), whereas BRL50481 was released slightly faster than Rolipram (Figure 8A, C). After four weeks the nanoparticles started to aggregate, demonstrated by measuring NP size and pdi (Figure 9). Since absorbance spectra depend on NP size and the aggregation of the particle led to a shift of the

absorbance spectra in a similar fashion, analysis at later time points is unfeasible (12). The reasons for the difference in drug (Rolipram, BRL50481) and dye (DiI, DiO) uptake and release between Rolipram and BRL50481 are not defined yet.

8.c. (Month 10-18) *Determine the protein adsorption on the NPs by incubation with fluorescent proteins.*

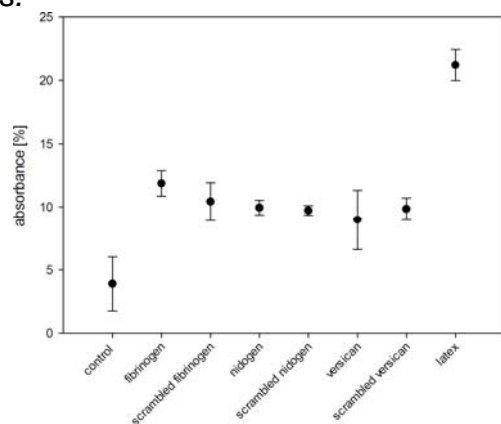


Figure 10: The PEG chain prevents protein adsorption to NP. The different types of NP were incubated with bovine serum albumin (BSA) and the amount of NP that stuck to the BSA was estimated by measuring the absorbance of the washed NP-BSA mix.

To determine the amount of protein adsorption on the different types of NPs, they were incubated with BSA and the absorbance measured. Non-functionalized NP showed the least amount of protein adsorption (4%), due to the dense corona of PEG chains. Peptide functionalized NPs were more prone to protein adsorption (9-12%), probably due to the peptide sequence (Figure 10). However naive as well as peptide-functionalized NP had a lower ability to bind proteins on its surface when compared to latex beads (21%, positive control, measured by autofluorescence).

8.d. (Month 13-18) *Organize obtained chemistry data and discuss final formulations before proceeding*

After obtaining the chemistry data, the different tNPs and appropriate scrambled peptide controls (PEG, 25%) were prepared in large quantities for in vivo studies.

8.e. (Month 13-24) *Prepare significant quantities of the different types of drug laden tNPs for in vivo testing [Note: tasks 8a-c will be repeated for each drug with sequential stock production to allow for in vivo testing to take place]*

Significant amounts of each drug-laden tNP were prepared and shipped in two batches for the in vivo studies.

Milestone 3: Prepared drug laden tNPs for in vivo efficacy testing after EAE

The drug laden tNPs were made, characterized and shipped for in vivo evaluation following EAE.

Study Objective 4: Screen drug laden tNPs for in vivo tissue release kinetics as well as anatomical and functional efficacy after EAE [Pearse]

Task 9 (Month 13-18): *Test drug laden tNPs in EAE to optimize tissue delivery and activity*

9.a. *Purchase and acclimatize animals*

9.b. *Induce EAE in the animals and provide drug encapsulated tNPs (or unmodified NP controls) systemically or directly at the time of first protein production/presence (earliest will be 2 h)*

9.c. *Perfuse animals and dissect tissues at 24 h, 3 d, 1 or 3 wk post-infusion*

9.d. Quantify PDE inhibitor drug levels (HPLC), PDE activity (RIA) and/or interferon-1 α (immunoblot) levels at the site of the demyelination lesion

9.e. Identify those tNPs that did not deliver therapeutic levels of the drugs to the site of CNS demyelination for the 3 wk period and re-formulate in vitro (e.g. increase drug load) for re-testing in vivo

9.f. Re-test any reformulated tNPs

Animals were ordered to evaluate the spinal cord penetration of drug and target enzyme effects after EAE when the different drug-laden tNPs were intravenously infused versus appropriate unloaded and scrambled peptide controls. In these studies, animals received tNPs beginning at 5 d post-EAE induction i.v. Animals were then evaluated behaviorally using a 0-6 point locomotor deficit score according to the following criteria - 0: no disease; 0.5: distal limp tail; 1: limp tail; 2: mild paraparesis, ataxia; 3: moderate paraparesis, the rats trips from time to time; 3.5: one hind limb is paralyzed, the other moves; 4: complete hind limb paralysis; 5: complete hind limb paralysis and incontinence; 6: moribund, difficulty breathing, does not eat or drink, euthanize immediately. At study endpoint animals were perfused or dissected to allow the examination of histopathology, drug penetration and enzyme targeting. Although animal numbers were not powered to look at behavioral effects of tNPs, all tNP groups showed a reduction in the degree of locomotor deficits (Figure 10), with the fibrinogen tNPs showing the most pronounced effects. Dil-laden fibrin tNPs had shown the greatest amount of spinal cord penetration in earlier work (Figure 1). Biochemical and histopathological analyses is ongoing.

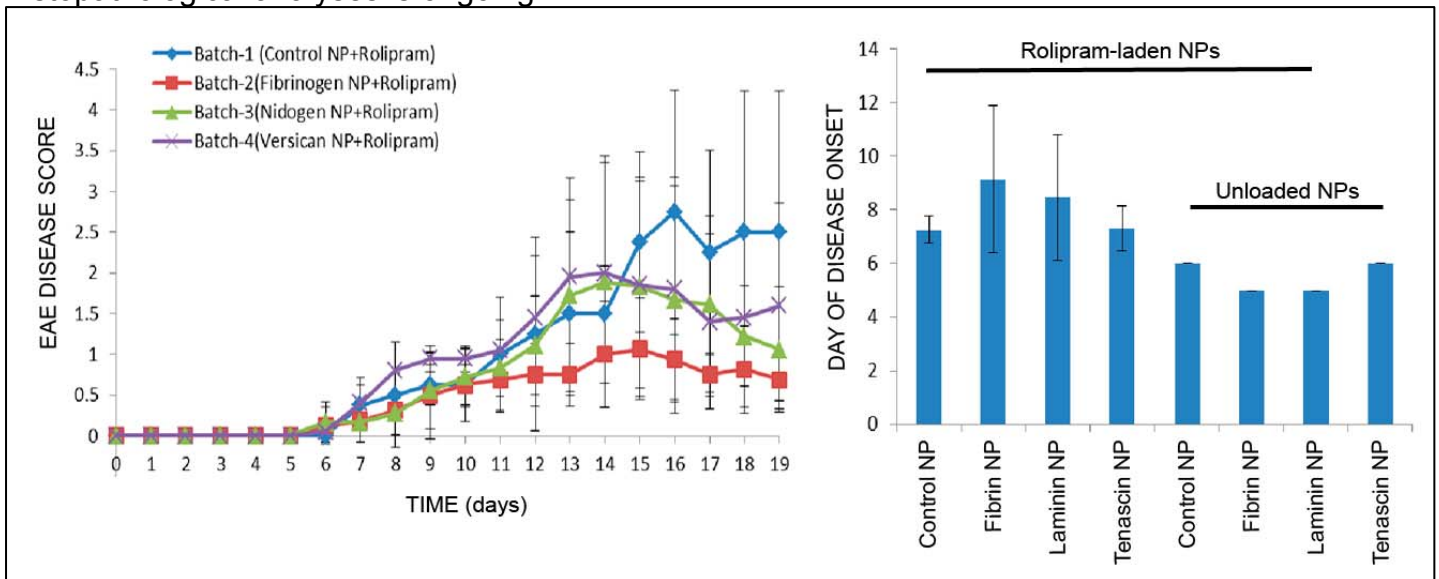


Figure 10: Animals receiving infusions of Rolipram-laden tNPs appear to have a reduced severity of disease after EAE induction compared to control, Rolipram-laden NPs (blue line). This was particularly evident with fibrinogen tNPs (red line). Similarly the day of first disease onset appeared delayed in those animals receiving tNPs with Rolipram compared to unloaded, control NPs.

Task 10 (Month 16-24): Test the efficacy (anatomical and functional) provided by tNPs (PDE inhibitors, interferon-1 α) vs. naked drugs or NP controls after EAE

10.a. Acclimatize, train and perform baseline functional testing for sensorimotor (catwalk, gridwalk) function

10.b. Induce EAE in the animals and provide drug encapsulated tNPs (or controls) systemically

10.c. Perform post-injury behavior analyses on the animals for up to 8 weeks

10.d. Obtain behavioral data and perform statistical analysis to look for significant differences between treated and untreated control groups

10.e. Perfuse animals and dissect tissues

10.f. Obtain fixed tissue, section tissue and examine inflammation, axon demyelination/injury and remyelination repair using immunostaining for specific markers or electronmicroscopy

10.g. Compare statistically the results between treated and controls

10.h. Put together a report summarizing the major findings of the behavioral and histological studies with graphs etc.

The drug-laden tNPs for this comparative study were received and experimental studies in animals are ongoing. Data from these investigations will be presented in the Final Report.

Milestone 4: Identified an effective drug-laden tNP strategy for therapeutic application to RRMS

We have shown that Rolipram-laden tNPs functionalized with peptides recognizing fibrinogen appear to retard disease progression in this experimental EAE model. Comparative studies that are underway will determine if this approach is more effective than naked drug or interferon-1a therapy.

Task 11 (Month 22-24): Finalize study results

11.a. Compile all results of the study into a final report

11.b. Submit any meeting abstracts, manuscripts or patents

11.c. Prepare a proposal for additional pre-clinical work and the initiation of IND paperwork for requesting a Phase 1 safety study in RRMS patients with the optimal therapeutic approach

Upon completion of the remaining in vivo investigations, the project data will be organized and a manuscript(s) prepared to report the findings of the work in a peer-reviewed journal(s). The data will also be used to seek further funding for pre-clinical studies that will drive this technology towards clinical implementation.

Milestone 4: Publication of findings

The work performed to date has resulted in three scientific meeting abstracts and manuscript(s) will be prepared upon the completion of the remaining in vivo studies to report the findings of these investigations.

Summary of Problems encountered for Year 2.

- In addition to delays in personnel hiring discussed and other problems discussed in the Year 1 annual report, in Year 2, a turnover of staff at Miami and the need to hire of a new technician delayed the in vivo work approximately 2-3 months.

KEY RESEARCH ACCOMPLISHMENTS (TO DATE)

- Nanoparticle formation with poly(ethylene glycol-b- ϵ -caprolactone) blockpolymer (PEG-b-PCL)
- Simplification of the nanoparticle preparation process
- Labeling of nanoparticles with fluorescent dyes
- Aminated (PEG-b-PCL) integration into (PEG-b-PCL) nanoparticles
- Peptide functionalization of aminated nanoparticles
- Improved fibrin gel retention with functionalized nanoparticles, compared to standard PEG-b-PCL nanoparticles
- Temporal evaluation of histopathological and behavioral deficits in an experimental EAE model that mirrors RRMS
- Temporal characterization of important ECM proteins and their natural ligands within the CNS in an experimental RRMS model
- Demonstration that tNPs (for laminin and fibrinogen) can be targeted to CNS lesions after EAE (feasibility of the technology)
- Demonstration that Rolipram-laden tNPs (for fibrinogen) appear to retard disease progression in an experimental EAE model (effectiveness of the technology)

REPORTABLE OUTCOMES

In Year 2, work from these investigations was presented at poster sessions/talks at The World Biomaterials Conference, The FASEB SRC Translational Neuroimmunology: From Mechanisms to Therapeutics Conference and The Zing Neurodegeneration Symposium.

CONCLUSION (summarizes total work to date)

Nanoparticles could be successfully generated from poly(ethylene glycol-b- ϵ -caprolactone) blockpolymer. NP had a size range of 28-36 nm when polymer or solvent concentrations were changed. Optimal nanoparticle formation in regard to size distribution was determined as 28.57% DMF and 3 mg polymer concentrations. NP allowed inclusion of fluorescent dyes (Dil, DiO) and aminated PEG-b-PCL. The peptides (i.e. NQEQVSP, DPEAAE and NIDPNAV) could be conjugated to the aminated PEG-b-PCL and integrated into the nanoparticles. Peptide (e.g. NQEQVSP and NIDPNAV and their scrambled controls) functionalized NP (fNP) was slightly larger in size than non-functionalized NP (range 30-42 nm). Factor XIIIa (NQEQVSP) fNP showed a better adherence than non-functionalized NP in fibrin gels, with 10% and 25% functionalization demonstrating the best adherence. NP and fNP had no surface charge and a low polydispersity index. Nanoparticles, surface functionalized with the peptides NQEQVSP and NIDPNAV (10% and 25%), were shipped to Miami to further test the binding of the peptide functionalized nanoparticles *in vivo*.

Initial *in vivo* work characterized temporal histopathological changes, behavioral deficits in locomotor function and protein production and localization of target ECM as well as their natural ligands in a specific EAE model that mirrors RR-MS; all these features were prominent by 10 days post EAE induction. Ensuing experiments investigated the accumulation of non-functionalized as well as laminin and fibrinogen tNPs at lesions within the lumbar spinal cord when administered systemically and repetitively just prior to and during the appearance of these disease features. It was demonstrated that laminin tNPs, but not the non-functionalized NPs or the first formulation of fibrinogen tNPs, accumulated at the site of CNS lesions. Subsequent studies with a new formulation of fibrinogen, but not tenascin-c, NPs showed accumulation within spinal cord tissues after EAE induction.

Following the development of functionalized NPs, *in vitro* studies examined drug loading of the particles. PEG-b-PCL NPs were able to be loaded with Dil, Rolipram and BRL-50481 as well as a combination of the agents. The release kinetics showed an initial bulk release of the incorporated drug when placed in solution followed by a smaller, sustained release for up to 28 days. When used *in vivo* following EAE, infusion of Rolipram-laden tNPs, particularly those functionalized with binding peptides for fibrinogen, reduced the severity of disease as well as delayed disease onset compared to control NPs. Ongoing studies will examine the use of the other drug-laden tNPs, determine the spinal cord penetration of the drugs delivered by this novel method as well as measure the effectiveness of drug-laden tNPs to provide anatomical and locomotor function improvements in an experimental model of RRMS.

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